18 May 1946.

Dear Francis,

How did the Colloquium go? Now that it has gone, perhaps I shall be hearing exciting news from you about coli reverse-mutations. Still have no further triple mutants, but may have soon. Also, what about doubles in the 'Coli B' strain where susceptibilty to any of the B viruses can be used as the genetic marker? For various reasons, I am planning to do much of the mutation work in this strain, but as yet have no mutations.

Are you familiar with Lincoln and Gowen's work on Phytomonas? They have a paper in Genetics, 27: 441 1942 'Mutation of P. stewartii by X-Ray irradiation' which is of considerable interest. They worked mainly with morphological characters, but they seem fairly clear cut. There, the incidence of multiple mutations was much greater than predictable from that of the single mutations, although the same qualification may hold as for the multiple resistances picked up by Demerec and Fano in their study of mutation to virus resistance: that these may be pleiotropic effects. Such an argument could not reasonably hold for these nutritional reversions. Ed tells me that the incidence of coincidental mutations in the Neurospora series was that expected on the basis of the singles. This stuff is of the very highest importance. If you would run a single experiment <u>on any material available</u> with results indicating such a phenomenon here, I'd be happier about putting in a fair amount of effort in getting a series of different multiples; otherwise it would not be worth the considerable investment. One can determine the number of prototrophs in the same plate as a determination of one mutant by plating into minimal, and later adding to the surface an excess of, say, threonine. This requires

covering the initial plate with a layer of unseeded agar. As for identification of any colonies that do come up, lactose fermentation and Gram stain allow for some assurance (that one has coli, at any rate.)

The time of my talk has been set tentatively for June 12. Can you make it?

Have some fairly cleancut experiments that just about tie up 'syntrophism+' There are critical levels of substrates- e.g. in the system Biotin-Methioninless + Threonine-Prolineless, + excess Biotin and Threonine, there is a threshold between .1 and .3 ug/10 ml of methionine that has to be added. The growth response is all or none. Apparently, the .3 ug of methionine allow enough growth of its homologous cells that they secrete enough proline, etc., This amount of growth is the least visible turbidity. On the other hand, analyses of wild type filtrates (even when grown with excess of various precursors as anthranilic ac., glutamic acid) is disappointing, as only traces (but definite traces) of growth factors are present in the medium. However, a more detailed study, with samples taken at different times in growth has to be undertaken. No evidence yet of recombination.

Heard from McClintock which strains to use for cytology (Chilton # Emerson) and will try same in hopes of incorporating factors in standard isogenic stocks. These are already at F-3 , so perhaps some time has been wasted.

No other news here; what's new with you? Regards to Betty and Lil,

Sincerely yours,